POLLEN TETRAD PATTERNS IN *LESCHENAULTIA*.

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(Plate x.)

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Synopsis.

Mature pollen grains of *Leschenaultia* remain associated in tetrads. In eleven collections from both wild and cultivated plants of three species, aborted pollen grains, varying in frequency from 7% to 53%, have been observed. All combinations of good and aborted grains in a tetrad were present in every collection. Univalent formation at meiosis has been observed. An hypothesis relating univalent distribution and pollen abortion patterns is presented. This, almost certainly, accounts for a large proportion of pollen abortion, but there may be other contributory causes.

INTRODUCTION.

The genus *Leschenaultia* R.Br. is different from other genera of the family Goodeniaceae in that the mature pollen grains remain in tetrads. This is uncommon, but not rare, among Angiosperms, but, whereas there is a tetrahedral arrangement of pollen grains in most such genera, in *Leschenaultia* there is a rhomboidal arrangement in a single plane. Mature pollen of *Leschenaultia* was stained with a cytoplasmic stain and it was observed that there was always a proportion of unstained, aborted pollen grains. Within tetrads every possible combination of stained and unstained pollen was observed, viz:

Full tetrads ..	4 stained
Triads	3 stained, 1 aborted
Dyads A	2 stained, 2 aborted, the latter being adjacent
Dyads O	2 stained, 2 aborted, the latter being opposite
Monads	1 stained, 3 aborted.
Nullads	4 aborted

These are illustrated in Plate x, figs. 1 and 2.

This paper is concerned with observations of the frequencies of the different types and a consideration of hypotheses to explain the observations.

MATERIALS AND METHODS.

Samples of mature pollen from plants of *L. formosa* R.Br. and *L. linarioides* DC. were collected by Mr. D. E. Symon in their native habitat in Western Australia. Most observations were carried out on plants of *L. formosa* and *L. biloba* Lindl. grown in cultivation. The samples of *L. linarioides* and *L. formosa* collected in their native habitat were diploid (2n = 18). Among cultivated plants of both *L. formosa* and *L. biloba*, diploids (2n = 18) and tetraploids were found. All the observations reported refer to diploid plants. It is worth recording that, whereas nearly all pollen produced by the tetraploid *L. formosa* plant aborted, the tetraploid *L. biloba* plant produced approximately 70% "fertile" (i.e. normally stained) pollen.

For mature pollen observations, buds were collected just before anther dehiscence and, after opening the corolla, fixed in acetic-alcohol (1:3). Anthers were dissected in a dextrin-sorbitol fluid similar to that described by Zirkle (1940) in which acid fuchsin replaced carmine. Aborted pollen grains were quite colourless and those assumed to be fertile stained bright red so that there was no doubt in distinguishing them. Little

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pollen was lost in mounting and tetrads were rarely broken or lying on their sides. All tetrads on a slide were counted and it is thought that errors in counting were negligible.

For observations of meiosis, buds were fixed in acetic-alcohol (1:3) and subsequently stored in 70% alcohol. Following hydration, buds were hydrolysed for five minutes in N HCl at 60°C. and then squashed in 2% synthetic orcein (G. T. Gurr, London) in 60% acetic acid. The pre-treatment with HCl not only improves the stain but helps separate the pollen mother cells, which otherwise tend to adhere to the tapetum. Alternatively an aceto-carmine squash technique was used without previous hydrolysis.

RESULTS.

1. *Observations on Pollen Grains.*

Frequencies of the different types of tetrads and the frequencies of aborted pollen grains are shown in Table 1.

TABLE 1.
Frequencies of Tetrad Types in Various Collections.

Species.	Plant and Collection.	Sample Size.	Full Tetrads.	Triads.	Dyads A.	Dyads O.	Monads.	Nullads.	Tetrads with at Least One Aborted Pollen Grain.	Percentage Aborted Pollen Grains.
L. linarioides ..	Wild plant 1	1,652	0·815	0·073	0·064	0·012	0·027	0·009	0·185	12·1
L. biloba ..	Cultivated plant 1, Collection 1	297	0·094	0·189	0·306	0·037	0·239	0·135	0·906	53·3
	Cultivated plant 1, Collection 2	349	0·185	0·421	0·248	0·058	0·072	0·016	0·815	40·3
	Cultivated plant 1, Collection 3	2,345	0·554	0·258	0·116	0·013	0·027	0·032	0·446	18·1
L. formosa ..	Wild plant 1	1,405	0·809	0·122	0·037	0·009	0·008	0·015	0·191	7·4
	Cultivated plant 1	970	0·911	0·041	0·022	0·014	0·003	0·009	0·089	3·9
	Cultivated plant 2 ..	1,658	0·589	0·293	0·078	0·014	0·013	0·013	0·411	14·2
	Cultivated plant 3 ..	703	0·341	0·404	0·159	0·033	0·037	0·026	0·659	24·7
	Cultivated plant 4 ..	934	0·358	0·442	0·140	0·023	0·022	0·015	0·642	27·4
	Cultivated plant 5, Collection 1	933	0·250	0·399	0·260	0·024	0·044	0·023	0·750	29·6
	Cultivated plant 5, Collection 2	811	0·361	0·430	0·136	0·023	0·035	0·015	0·639	22·8

Although there is considerable variation in their frequencies, abortion patterns are similar in all three species whether collected in cultivation or the native state. Thus the underlying causes of abortion may be similar in all three species and must be present in the native state.

The great variability between the three different collections from the one plant of *L. biloba* suggests that the environment has an important effect. This is confirmed by the two collections from the fifth cultivated plant of *L. formosa*. These were statistically different (χ^2 for heterogeneity = 51·818 with 3 degrees of freedom. P < ·001).

However, within a bud the contents of anthers are not heterogeneous. The individual counts for the five anthers from the one bud of *L. formosa* wild plant 1 are shown in Table 2.

This result has been confirmed using buds from *L. formosa* cultivated plants 3, 4 and 5, the probabilities derived from the contingency χ^2s being 10–20%, 10–20% and 60–70% respectively.

These data may be summarized as showing that there may be a large variation between buds on the same plant, but that the anthers within a bud are homogeneous.

The large variation within a plant made it difficult to correlate observations on meiosis and on pollen grains. In the absence of facilities for growing plants under constant environmental conditions, collections of meiotic material of *L. biloba* were

made and other buds, of similar length and presumably at the same stage, were marked for later pollen grain studies. Only one pair of collections was successful at both stages. The pollen grain counts are shown in Table 3. The three buds were not heterogeneous, which lends support to the assumption that they were at the same stage when marked.

In some tetrads one or more small aborted microcytes were observed (Plate x, fig. 3).

TABLE 2.

Numbers of Tetrad Types in One Bud of L. formosa *(Wild Plant 1).*

Anther.	Full Tetrads.	Triads.	Dyads A.	Dyads O.	Monads.	Nullads.	Total.
1	239	41	10	2	2	3	297
2	219	38	7	3	1	2	270
3	219	35	16	0	2	8	280
4	225	27	14	7	4	5	282
5	234	31	5	1	2	3	276
Total ..	1,136	172	52	13	11	21	1,405

χ^2 heterogeneity $= 19 \cdot 836$ with 12 degrees of freedom* $0 \cdot 10 > P > 0 \cdot 05$.
* Dyads O, Monads and Nullads were grouped into one class.

TABLE 3.

Numbers of Tetrad Types in Three Buds of L. biloba *(Plant 1, Collection 3) Judged to have been at the Same Stage of Development.*

Bud.	Full Tetrads.	Triads.	Dyads A.	Dyads O.	Monads.	Nullads.	Total.
1	465	247	103	14	27	26	882
2	439	185	78	8	14	21	745
3	396	172	90	9	22	29	718
Total ..	1,300	604	271	31	63	76	2,345

χ^2 heterogeneity $= 13 \cdot 233$ with 10 degrees of freedom $0 \cdot 20 < P < 0 \cdot 30$.

2. Observations on Pollen Mother Cells.

In all three species the diploid complement of chromosomes was 18. Detailed studies of meiosis were confined to *L. formosa* and *L. biloba*. Prophase stages were difficult to observe. At metaphase I, a large proportion of bivalents was seen to have a single terminalized chiasma (Plate x, fig. 4). In *L. biloba* the chiasma frequency in one sample was $9 \cdot 85 \pm 0 \cdot 95$ and in a sample from *L. formosa* $11 \cdot 55 \pm 0 \cdot 3$.

Univalents were frequently observed at metaphase I, anaphase I and early telophase I (Plate x, fig. 5). The frequencies for *L. biloba* (cultivated from plant 1) are shown in Table 4.

Of 780 cells scored, 100 contained univalents, 98 cells with one pair and two cells with two pairs. Similar observations in three collections from *L. formosa* showed univalents to be present in $10 \cdot 6\%$, $17 \cdot 4\%$ and $11 \cdot 4\%$ of pollen mother cells respectively.

Collection 3 in Table 4 corresponds to the pollen grain data shown in Table 3, i.e. the buds in Table 3 were judged to have been undergoing meiosis at the same time as Collection 3, Table 5, was made. The important point to be noticed is that. whereas univalents appeared in only 23% of pollen mother cells, aborted pollen grains appeared in 44·6% of tetrads.

Although frequencies have not been estimated, laggards have also been observed at telophase II (Plate x, fig. 6). It was also observed in cells at the second division of meiosis that spindles were always parallel and never crossed. An important implication of this is that, in a dyad–O tetrad, a stained and an aborted cell must be sister cells

from the same second division spindle. When each spindle gives rise to one stained and one aborted pollen grain, usually equal numbers of dyad–A and dyad–O tetrads should result. Dyad–A, but not dyad–O, tetrads could also result from some polar inequality at the first division of meiosis.

TABLE 4.

Number of Pollen Mother Cells of L. biloba *Containing Univalents at Metaphase 1— Telophase 1.*

Collection.	Cells with Univalents.	Total Cells.	Percentage of Cells with Univalents.
Collection 1—			
Bud 1	27*	273	10
Bud 2	18	161	11
Collection 2	20*	195	10
Collection 3	35	151	23
Total	100	780	—

* Includes one cell with four univalents.

DISCUSSION.

The data in Tables 1, 2 and 3 show that, when buds, from one plant, at different stages of development were studied, the tetrad patterns were heterogeneous. However, when buds at approximately the same stage of development were studied, the tetrad patterns were not heterogeneous. Because anthers in a bud also were homogeneous it is deduced that the environment of the plant is important in determining the frequency of pollen abortion.

One simple hypothesis which should be considered to account for the abortion patterns observed is that abortion was caused by a randomly occurring event. If this were so, the frequencies of the different patterns should have followed a binomial distribution. Thus in the data shown in Table 2, the frequency of aborted grains was $\frac{414}{5620} = \cdot0744$. The expansion of $(\cdot9256 + \cdot0744)^4$ should give the expectations in the different classes of tetrads. These are as follows.

	Full Tetrads.	Triads.	Dyads.	Monads.	Nullads.
Expected	1030·4	332·2	40·2	2·1	Very small
Observed	1136	172	65	11	21

Also the frequency of adjacent dyads should have been twice that of opposite dyads. The observations were 52:13. There were significant departures from both expectations and these were also shown by all other data. On its own, at least, this hypothesis must be discarded.

The high frequency of univalents observed at meiosis suggests that pollen abortion might be explained by their distribution. The low frequency of cells with more than one pair of univalents (observed twice in 780 cells) suggests that an hypothesis could be formulated taking account only of cells with one pair of univalents.

Let p be the frequency of formation of one pair of univalents; i.e. 1–p is the frequency of non-formation of univalents. Any one univalent may have one of three fates, i.e.:

(1) Be included undivided at one pole at anaphase I. Let the frequency of this be x.

(2) Be lost, i.e. not included at either pole at anaphase I. Let the frequency of this be y.

(3) Divide at anaphase I, one chromatid going towards each pole. Let the frequency of this be z.

Misdivision may be included with loss since the result would be the same. Thus $x + y + z = 1$ .... Equation 1.

When a univalent divides, an individual chromatid may be lost, either before telophase I or during the second meiotic division. Let the frequency of this, for any one chromatid, be w.

Using these parameters, the frequencies of the different tetrad types have been worked out and are shown in Tables 5 and 6. Table 5 is based on the additional hypothesis that deficiency only leads to abortion, i.e. disomic pollen grains are viable. Table 6 is based on the alternative hypothesis that any unbalance leads to abortion.

The six equations derived from either table, together with Equation 1, could, in theory, be solved by the method of maximum likelihood (Mather, 1951, page 47) for any particular set of data. The task is, however, formidable and has not been achieved.

TABLE 5.

Tetrad Types Resulting from Distribution of Univalents on the Hypothesis that Only Deficiency Leads to Abortion. For further explanation, see text. To distinguish the two poles, they are called N and S. For brevity, " full tetrads " are shown as " tetrads ".

1st univalent / 2nd univalent	Lost.	Included Whole.		Divided and Included.				Frequency.
		N.	S.	N & S.	N.	S.	Nil.	
Lost	Nullad	Dyad A	Dyad A	½ Dyad A ½ Dyad O	Monad	Monad	Nullad	y
Included whole — N	Dyad A	Dyad A	Tetrad	Triad	Dyad A	Triad	Dyad A	$\dfrac{x}{2}$
Included whole — S	Dyad A	Tetrad	Dyad A	Triad	Triad	Dyad A	Dyad A	$\dfrac{x}{2}$
N & S	½ Dyad A ½ Dyad O	Triad	Triad	¼ Tetrad ½ Triad ⅛ Dyad A ⅛ Dyad O	½ Triad ¼ Dyad A ¼ Dyad O	½ Triad ¼ Dyad A ¼ Dyad O	½ Dyad A ½ Dyad O	zw²
Divided and included — N	Monad	Dyad A	Triad	½ Triad ¼ Dyad A ¼ Dyad O	½ Dyad A ½ Dyad O	½ Dyad A ½ Dyad O	Monad	zw(1−w)
Divided and included — S	Monad	Triad	Dyad A	½ Triad ¼ Dyad A ¼ Dyad O	½ Dyad A ½ Dyad O	½ Monad ½ Dyad A	Monad	zw(1−w)
Nil	Nullad	Dyad A	Dyad A	½ Dyad A ½ Dyad O	Monad	Monad	Nullad	z(1−w)²
Frequency	y	$\dfrac{x}{2}$	$\dfrac{x}{2}$	zw²	zw(1−w)	zw(1−w)	z(1−w)²	

Total frequencies :

Full tetrads $= (1-p) + p(\tfrac{1}{2}x^2 + \tfrac{1}{4}z^2w^4)$.

Triads $= pzw[2x + zw^2(2 - 1\tfrac{1}{2}w)]$.

Dyads A $= px[2y + 2z(1-w) + \tfrac{1}{2}x] + pzw^2\{y + z[\tfrac{1}{2}w^2 + (1-w)(3-2w)]\}$.

Dyads O $= pzw^2[y + \tfrac{1}{2}zw^2 + z(1-w)(2-w)]$.

Monads $= pzw(1-w)[4y + z(1-w)(4-3w)]$.

Nullads $= py^2 + pz(1-w)^2[2y + z(1-w)^2]$.

If it were done the whole univalent hypothesis and the alternative subsidiary hypotheses could be tested.

Observations of univalents at meiosis I, of laggards at meiosis II and of microcytes within spores showing abortion patterns support the univalent hypothesis. Microcytes presumably form around the univalents. The frequency of tetrads containing one or more aborted spores should be less than the frequency of pollen mother cells containing univalents because a full tetrad may be formed even when univalents are present at meiosis (Tables 5 and 6). In the observation reported here, the frequency of tetrads containing one or more aborted spores ranged from 9% to 90% with a mean of 52%;

<div align="center">TABLE 6.</div>
Tetrad Types Resulting from Distribution of Univalents on the Hypothesis that any Unbalance Leads to Abortion.
For further explanation, see text. To distinguish the two poles, they are called N and S. For brevity, " full tetrads " are shown as " tetrads ".

1st univalent / 2nd univalent	Lost.	Included Whole.		Divided and Included.				Frequency.
		N.	S.	N & S.	N.	S.	Nil.	
Lost	Nullad	Dyad A	Dyad A	½ Dyad A ½ Dyad O	Monad	Monad	Nullad	y
Included whole — N	Dyad A	Nullad	Tetrad	½ Dyad A ½ Dyad O	Monad	Triad	Dyad A	$\frac{x}{2}$
Included whole — S	Dyad A	Tetrad	Nullad	½ Dyad A ½ Dyad O	Triad	Monad	Dyad A	$\frac{x}{2}$
Divided and included — N & S	½ Dyad A ½ Dyad O	½ Dyad A ½ Dyad O	½ Dyad A ½ Dyad O	¼ Tetrad ¼ Nullad ½ Dyad A	½ Monad ½ Triad	½ Triad ½ Monad	½ Dyad A ½ Dyad O	zw^2
Divided and included — N	Monad	Monad	Triad	½ Monad ½ Triad	½ Dyad A ½ Nullad	½ Dyad A ½ Dyad O	Monad	$zw(1-w)$
Divided and included — S	Monad	Triad	Monad	½ Triad ½ Monad	½ Dyad A ½ Dyad O	½ Dyad A ½ Nullad	Monad	$zw(1-w)$
Divided and included — Nil	Nullad	Dyad A	Dyad A	½ Dyad A ½ Dyad O	Monad	Monad	Nullad	$z(1-w)^2$
Frequency	y	$\frac{x}{2}$	$\frac{x}{2}$	zw^2	$zw(1-w)$	$zw(1-w)$	$z(1-w)^2$	

Total frequencies :

Full tetrads $= (1-p) + p(\frac{1}{2}x^2 + \frac{1}{4}z^2w^4)$.

Triads $\quad = 2pzw(1-w)(x+zw^2)$.

Dyads A $\quad = pzw^2[x+y+z(\frac{1}{2}w^2+3(1-w)^2)] + 2px[y+z(1-w)^2]$.

Dyads O $\quad = pzw^2[x+y+2z(1-w)^2]$.

Monads $\quad = 2pzw(1-w)\{x+2y+z[w^2+2(1-w)^2]\}$.

Nullads $\quad = pz(1-w)^2\{2y+z[w^2+(1-w)^2]\} + p(y^2+\frac{1}{4}zw^2+\frac{1}{2}x^2)$.

the frequency of pollen mother cells containing univalents ranged from 10% to 23% with a mean of 13%. Although there is a considerable difference between these mean values, this cannot be taken as disproof of the univalent hypothesis because of the great variability and the overlapping distributions. Moreover, with one exception, there was no direct correlation between the two sorts of observations. In the one set of data in which an attempt was made to estimate frequencies of tetrad types which could be correlated with meiotic observations, tetrads with aborted spores were twice as frequent as pollen mother cells with univalents. This suggests that univalent distribution alone

can account for only part of the observed abortion, but it is not a rigorous enough test to exclude its accounting for all abortion.

If univalent distribution cannot account for all abortion, there must be other causes co-existing, e.g., abortion due to randomly occurring events would increase the frequency of triads and dyads, but would make less difference to the frequencies of monads and nullads. A further hypothesis which should be mentioned is that proposed, for some species (e.g., *Astroloma pinifolium*) of the Epacridaceae by Smith-White in a paper in this Part of these Proceedings (pp. 259-270). If, at meiosis, the segregation of cytoplasmic and/or chromosomal factors is equational at the first division but reductional at the second, such as to cause abortion at both poles, one pole or neither in each half mother cell in the ratio x:y:z, then the different tetrad types can be derived from the trinomial $(x + y + z)^2$. Because univalent distribution is responsible, almost certainly, for a large proportion of abortion, no attempt has been made to apply the trinomial hypothesis alone. It is mentioned as a possible co-existing cause of abortion, although it is considered unlikely to be operating because terminal localization of chiasmata causes reduction at the first meiotic division for most chromosomal material.

It is not apparent how a rather high proportion of inviable gametes persists in these species. Presumably this selective disadvantage is outweighed by some contingent advantage. For example, the terminal localization of a single chiasma may preserve intact different combinations of genes, the presence of which is advantageous to a local population of species. The chance failure of formation of this chiasma would result in univalents and relative infertility. Possibly the disadvantage of this is not as great as the advantage of terminal localization of a single chiasma. This may be particularly true where there is always some fertile pollen and pollination is very efficient as it may be in *Leschenaultia* with its elaborate indusium for collecting and holding pollen.

Acknowledgements.

References.

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EXPLANATION OF PLATE X.

Fig. 1. Camera lucida drawings of the various types of tetrads in *Leschenaultia formosa.* Top (left to right): full tetrad, triad, dyad O; bottom (left to right): nullad, dyad O, monad, dyad A. × 200.

Fig. 2. *Leschenaultia formosa.* Pollen tetrads stained with acid fuchsin showing full tetrads, triad, dyad A and monad. × 100.

Fig. 3. *Leschenaultia biloba.* Pollen tetrads showing full tetrads, dyad O and dyad A, with one microcyte. × 100.

Fig. 4. *Leschenaultia biloba.* Pollen mother cells at metaphase I. In the majority of bivalents there is a single, terminally localized chiasma. Aceto-orcein; × 830.

Fig. 5. *Leschenaultia biloba.* Pollen mother cell at telophase I, showing two univalents. Aceto-orcein; × 830.

Fig. 6. *Leschenaultia formosa.* Pollen mother cell at telophase II showing laggards. Aceto-carmine; × 830.